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Leaf Analysis as a Guide to the Nutrition of Hevea brasiliensis I. Sampling Technique with Mature Trees: Principles and Preliminary Observations on the Variations in Leaf Nutrient Composition with Position on the Tree

V. M. SHORROCKS

The variations in leaf nutrient composition on mature Hevea between leaves exposed to full sunlight and shaded* leaves, and with height of sampling and aspect, were investigated. Differences between the nutrient content of leaves in the light and in shade, and with height of sampling of the shade leaves, were clearly demonstrated.*

Little difference was observed in the nutrient content of light leaves at different heights on the tree or in that of light leaves on the north and south aspects, and the sampling of leaves exposed to light on the top of the canopy is a method that incurs only a small sampling error. The principles of, and practical problems entailed in leaf sampling and its use as a diagnostic tool are discussed, and four alternative methods of leaf sampling for mature Hevea are briefly discussed.

Differences were found in the magnitude of the effects of fertilisers on the nutrient composition of leaves from different positions and preliminary results are discussed.

INTRODUCTION

Plant analysis techniques have been used successfully with many crops to determine fertiliser requirements (GOODALL and GREGORY, 1947; LEYTON, 1948), and recently such a technique for *Hevea*, based largely on leaf analysis, has been described (BEAUFILS, 1958a; 1958b; 1959). The basic problem involved in using leaf analysis as a guide to manuring is that of studying the relationship between the leaf nutrient content and the response obtained from fertiliser applications, in order that a forecast can be made, from a knowledge of the leaf nutrient content, of the response likely to be obtained from fertiliser applications under field conditions.

Sampling technique. The selection of a suitable technique of sampling is a first step in studying this problem. It is important to use a technique which has a small sampling error and involves the minimum expenditure of time and labour. It is possible however that a sampling technique with a large sampling error may prove to give a more accurate forecast than one with only a small sampling error, in which case a balance must be sought by increasing the number of trees to be sampled and widening the distribution of those trees, so as to minimise the error, without involving an excessive amount of labour.

Leaves were chosen as the organ of the tree to be sampled since from other work they are known to be sensitive indicators of the nutrient status of the plant, and also because extensive work has already been carried out on leaf nutrient composition by BOLLE-JONES (1954a; 1957a; 1957b) and BEAUFILS. Bark sampling has been considered, and employed on a limited scale, but was rejected because of the damage done to the tree and because

* These are referred to as 'light' and 'shade' leaves, respectively, throughout this paper.

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of the impossibility of carrying out repeated samplings on any one tree.

In carrying out leaf sampling it would be unwise to sample leaves taken from any arbitrarily chosen position in the absence of knowledge concerning leaf variability and sensitivity to nutritional changes. The present studies have been directed towards obtaining information on the variations in leaf nutrient composition with position on the tree so that a sampling method could be evolved which would incur the minimum sampling error compatible with effort involved.

Leaf age and stage of development. Leaf nutrient composition will vary considerably with age, and ideally only leaves that are all at the same stage of development should be sampled, as a first means of reducing the sampling error. The collection of leaves that are all at the same stage of development is relatively easy when the plant has a regular pattern of leaf formation as in cereals, oil palms and conifers, for it is possible to define in morphological terms particular leaves for sampling. With *Hevea* the problem is not as simple, but it is possible to define a whorl of leaves at a particular stage of development: namely a top whorl (or end whorl) on a branch with a dormant growing point, and from which whorl, and the whorl immediately below, there is no development of the axillary meristems. However this definition is by no means precise, as owing to the recurrent production of new whorls on mature *Hevea*, there are present on the tree at any given time in the year top whorls, such as those described above, which are of markedly different ages and stages of development.

The practical problems involved in marking whorls on a tree as they start to expand, for future sampling at a precise age, precludes the use of a technique that would allow leaves of exactly the same age to be sampled, as a routine. However, leaf age can be roughly ascertained by inspection of fresh leaves: young leaves are characteristically thin, light green, clean, quick to wilt and soft to the touch; old leaves show loss of bloom, and carry lichens and non-parasitic fungi on the laminae and petioles.

The sampling practice in the present study has been to eliminate both the old and the young leaves, and to collect only leaves from whorls that are considered to have hardened (matured) a short time before sampling. This is a subjective process and may introduce differences between samplers, but is has been adopted as the most practicable method possible.

Leaf position. The morphological position of the leaves to be sampled is a problem with *Hevea*, which carries leaves in the light and shade, and at different heights.

Two methods of sampling leaves from mature *Hevea* have been described previously (CHAPMAN, 1941; BEAUFILS, 1955). Chapman sampled leaves from the top of the canopy which were exposed to full sunlight, and were borne on a terminal branch carrying only one whorl of leaves. CHAPMAN (1941) named such branches 'spurs of limited growth', as they were similar to the fruiting spurs on apple trees, and he stated that terminal branches carrying two whorls of leaves were less numerous than spurs of limited growth. Beaufils adopted a method of sampling shaded leaves inside the crown, from short, thin branches borne on the main or primary branches and carrying only one whorl of leaves. He believed these to be the same leaves as those sampled by Chapman (BEAUFILS, 1955) and he named the branches, 'branches of limited growth', a term almost identical with that used by Chapman. Some confusion has resulted from the use of the same description for two distinct leaf types. For convenience the Chapman type of leaf will be called spur leaves (light) and the Beaufils type spur leaves (shade).

Both methods of sampling described above have their disadvantages. Collection of leaves from the top of the canopy by climbers is dangerous work, and if the trees are tall or weakly branched this task is often impossible. The latter situation immediately precludes large areas of *Hevea* from being sampled by this method. Furthermore, the actual posi-

tion in the crown from which the terminal branch is cut is selected by the climber, whose judgement may at times be unreliable.

The scarcity of spur leaves (shade) makes the second method of sampling difficult, and there is a great risk of leaves of unsuitable age being collected to make up the sample. On clone PB 86 in Malaya, for instance, relatively few spur leaves (shade) are to be found, and under such circumstances there is a danger that the few trees bearing such branches are not representative of the area under study. An experimental difficulty in this method is that it has been found impossible to maintain an adequate frequency of sampling in some manuring experiments, since an insufficient number of the spur leaves (shade) have been found in the comparatively small number of trees available.

Time of sampling. A small diurnal variation in leaf magnesium content, but not of nitrogen, phosphorous, potassium, calcium and manganese, has been reported by BOLLE-JONES (1957b), and CHAPMAN (1941) has also stated that diurnal fluctuations in leaf nutrient composition occur, although he gives no data. It is considered however that such variations are insufficient to introduce a large error and consequently time of sampling has not been taken into consideration in the work reported below. In practice practically all the leaf sampling has been carried out between 07.00 hours and 14.00 hours.

Owing to the surprising lack of published data on the differences between sampling techniques, it was felt that a study of this problem was required. The existence of differences in the nutrient composition of similarly aged leaves, related to position on the tree, is suggested by the fact that certain nutrient deficiency symptoms are only exhibited by leaves in a particular position and not by leaves all over the tree; magnesium and potassium deficiency symptoms on mature rubber are found on leaves at the top of the canopy, or on leaves that are exposed to full sunlight; manganese deficiency symptoms are usually observed low down in the tree on shade leaves: a papery scorch which is at present thought to be associated with calcium deficiency is also more prevalent on shade leaves low down in the tree.

The present investigation was designed to study the variations in leaf nutrient composition with position on the tree, and the opportunity was also taken to undertake a preliminary study of the effects of fertilisers on leaves from the different positions.

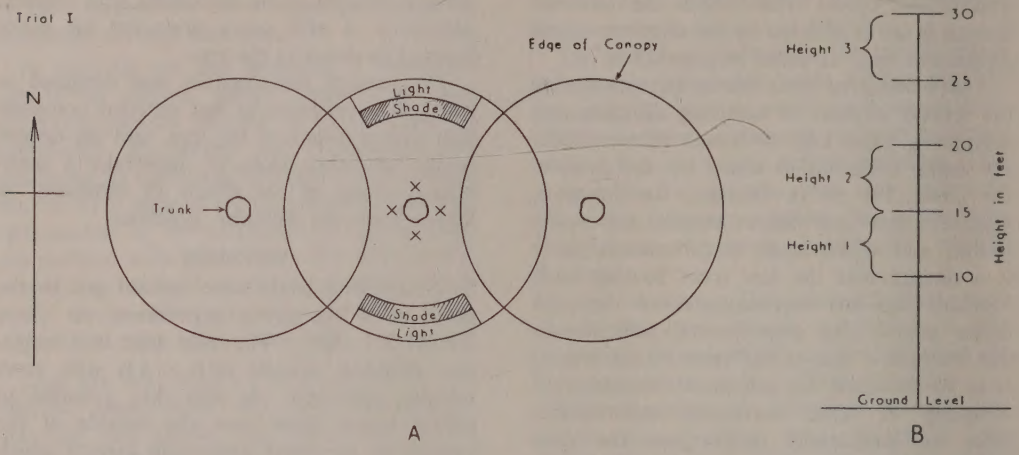
METHODS

Two sampling trials were carried out in the plots of a manuring experiment on clone RRIM 501 trees. The trees were in a hedgerow planting, spaced 60 ft × 4 ft with rows running east-west. It was thus possible to collect leaves from over the outside of the canopy on the north and south aspects which were exposed to full sunlight. The leaves from the top of the tree were also in full sun, but the leaves collected on the east and west aspects of a tree were shaded by the canopy of the adjacent tree and by the higher branches on the same tree.

Sampling Trial 1

This trial entailed the positional sampling of four trees in a plot which had received applications of ammonium sulphate, potassium chloride and rock phosphate. The sampling positions employed are shown in *Figure 1*. Leaves were collected from the lowest part of the canopy (height 1), half way up the side of the canopy (height 2), and at the top of the canopy (height 3), from whorls that had recently matured. From each whorl the basal four leaves (twelve leaflets) were taken.

Leaves exposed to full sunlight on the north and south aspects were collected at all heights, and an endeavour was made to collect shade leaves from the inner part of the canopy also on the north and south aspects at all heights. Such shade leaves, cut down by a man standing on a ladder just outside the canopy, were close to the edge of the canopy and were therefore only shaded to a very slight extent. Four discrete samples were thus collected at each height, and four samples of spur leaves (shade) were collected



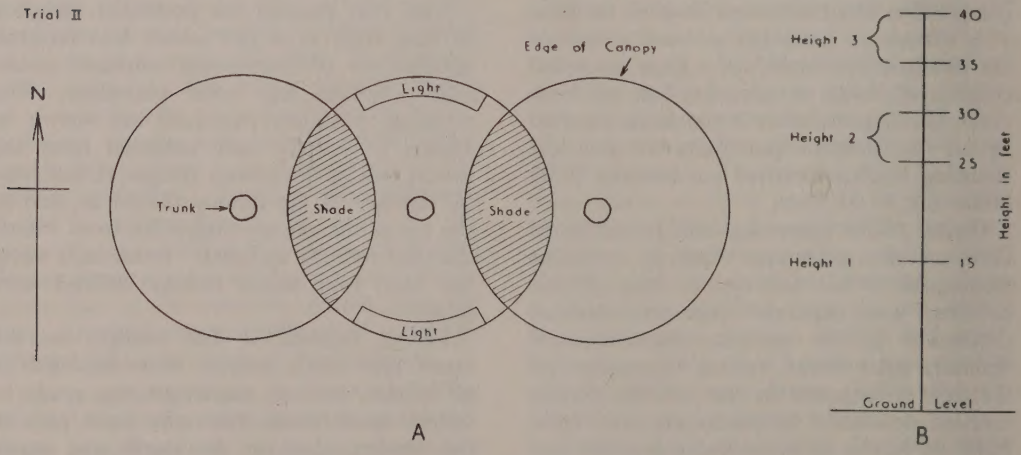
A. Leaves were collected from the North and South aspects:

i. On the outside of the canopy in full sunlight ... LIGHT

ii. On the inside of the canopy in the shade ... SHADE

In addition, four sets of spur leaves (shade) were collected from each tree (×)

B. The light and shade leaves were collected at three heights from the ground on both the North and South aspects.



A. Leaves collected from the North and South aspects were exposed to full sunlight on the outside of the canopy ... LIGHT

Leaves collected from the East and West aspects were shaded by the canopy of the adjacent tree and by higher branches on the same tree ... SHADE

B. Leaves were collected at three heights from the ground on all four aspects.

Figure 1. Leaf sampling positions: Trials I (above) and II (below).

from each tree. The sixteen samples from each tree were analysed separately.

Sampling Trial II

This trial was carried out on six plots of the experiment: two control plots, two plots receiving the highest level of ammonium sulphate and two plots receiving the highest level of potassium chloride were sampled (*Table 4*). Work had already shown that the bulk collection of light leaves from the top of the canopy of six trees in each plot of this experiment was satisfactory for detecting treatment effects; consistent effects of ammonium sulphate and potassium chloride on the leaf nitrogen, potassium and magnesium contents of mature leaves on the top of the canopy were observed (SHORROCKS, 1961). The plots were chosen in order that the study would cover a wider range in the leaf content of nitrogen, potassium and magnesium than would otherwise be obtained by sampling in one plot, and also to enable a preliminary assessment to be made of the relative sensitivity of the leaves from different positions in reflecting changes in tree nutrient content caused by fertiliser application.

Leaves were collected from twelve positions (three heights × four aspects, *Figure 1*), on six trees in each plot, the leaves from the individual positions being bulked to form twelve positional samples per plot. It is to be noted that all leaves collected from all heights on the north and south aspects were leaves on the outside canopy and were exposed to full sunlight, and that leaves collected from the east and west aspects were shade leaves. However the leaves collected from height 3 on the east and west aspects (shade leaves) were subjected to a lower degree of shading than leaves from height 1 as they were situated nearer the top of the canopy.

The leaf sampling for both trials was carried out at the same time of the year—Trial I (19.11.1957) and Trial II (21.10.1958). The preparation of the leaf samples and the analytical procedures employed have been described by BOLLE-JONES (1954b), and BOLLE-JONES, MALLIKARJUNESWARA AND RATNASINGAM (1957). The petioles and mid-ribs were discarded. The nitrogen, phosphorus, potassium, magnesium, calcium, sulphur, manganese, copper and iron contents were determined in both trials, and in Trial II the leaf boron, molybdenum and zinc contents, were also determined. All results are quoted in the tables as percentages or p.p.m. on a dry weight basis.

An analysis of variance was carried out for each element determined, and the appropriate standard errors of the positional means were calculated and used in the method of least significant difference to test for significance at the 5% level. Significant differences are only included in the tables where the mean values differ significantly. In testing for differences between the nutrient composition of light and shade leaves in Trial I the spur leaves (shade) data were not included because all such leaves were shade leaves (see *Figure 1*, Trial I, A), whilst for the comparison of leaf nutrient composition with height it was included.

RESULTS

Sampling Trial I

1. *Variations between light and shade leaves.* A comparison of the nutrient composition of the light and the partially shaded leaves indicated that the shade leaves contained a significantly higher calcium content (*Table 1*). This, however, has not been apparent in later, so far unreported, investigations. No significant differences between light and shade leaves was observed for the other nutrients. In view of other investigations, which show large differences between the composition of light and shade leaves, the result of this preliminary investigation is attributed to the fact that the shade leaves were only slightly shaded, as they were situated near the outside of the canopy.

2. *Variation due to height of sampling.* As only slight differences were observed between the nutrient composition of light and shade leaves, the variations in the nutrient composition with height are considered for both light and shade leaf data together

TABLE 1. TRIAL I. VARIATIONS IN NUTRIENT COMPOSITION OF LIGHT AND SHADE LEAVES, AND OF LEAVES SAMPLED FROM DIFFERENT HEIGHTS †

(An angular transformation was required in the statistical analysis of the calcium data, and the transformed values are in parenthesis)

	% K	% Mg	% P	% S	p.p.m. Cu	% N	p.p.m. Mn	% Ca	p.p.m. Fe
Light	0.86	0.20	0.18	0.20	13	3.64	32	0.52(4.13)	68
Shade	0.79	0.20	0.18	0.21	14	3.55	30	0.61(4.18)	71
s.e.	± 0.027	± 0.007	± 0.005	± 0.007	± 0.3	± 0.075	± 1.8	± (0.107)	± 1.5
Min. 5% sig. diff.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	(0.308)	N.S.
Spur leaves (shade)	0.96	0.27	0.19	0.24	18	3.74	29	0.51(4.09)	66
Height 1 (low) *	0.90	0.23	0.19	0.21	14	3.78	31	0.53(4.17)	69
Height 2 (medium) *	0.80	0.19	0.18	0.20	13	3.45	32	0.57(4.33)	67
Height 3 (high) *	0.79	0.18	0.18	0.19	13	3.56	30	0.59(4.40)	72
s.e.	± 0.040	± 0.011	± 0.005	± 0.010	± 0.7	± 0.102	± 2.2	± (0.154)	± 1.9
Min. 5% sig. diff.	0.115	0.032	0.015	0.029	2.0	0.290	N.S.	N.S.	5.4

* Shade and light leaves combined.

† Results expressed on a dry matter basis.

TABLE 2. TRIAL II. VARIATIONS IN NUTRIENT COMPOSITION OF LIGHT AND SHADE LEAVES, AND OF LEAVES COLLECTED FROM THE NORTH, SOUTH, EAST AND WEST ASPECTS OF THE TREE †

	% K	% Mg	% P	% S	p.p.m. Cu	p.p.m. Zn	% N	p.p.m. Mn	p.p.m. B	% Ca	p.p.m. Fe	p.p.m. Mo
Light	0.88	0.25	0.189	0.26	13.0	21.4	3.68	38	39.0	0.53	94	0.21
Shade	1.01	0.26	0.194	0.27	14.0	26.0	3.57	32	31.3	0.52	92	0.24
s.e.	± 0.012	± 0.004	± 0.0020	± 0.004	± 0.28	± 0.45	± 0.023	± 1.2	± 1.08	± 0.019	± 1.4	± 0.02
Min. 5% sig. diff.	0.034	0.011	N.S.	0.012	0.80	1.27	0.064	3.4	3.06	N.S.	N.S.	N.S.
North	0.90	0.26	0.192	0.26	13.4	22.2	3.73	40	36.5	0.54	93	0.19
South	0.87	0.24	0.187	0.25	12.5	20.6	3.62	36	41.5	0.52	96	0.23
East	1.02	0.26	0.192	0.27	13.6	26.2	3.55	34	31.7	0.50	92	0.23
West	1.00	0.26	0.196	0.28	14.6	25.9	3.58	31	30.9	0.53	92	0.25
s.e.	± 0.017	± 0.005	± 0.003	± 0.006	± 0.40	± 0.64	± 0.032	± 1.7	± 1.53	± 0.027	± 2.0	± 0.032
Min. 5% sig. diff.	0.049	0.015	0.008	0.018	1.13	1.80	0.091	4.8	4.32	0.076	N.S.	N.S.

† Results expressed on a dry matter basis.

TABLE 3. TRIAL II. VARIATIONS IN NUTRIENT COMPOSITION OF LIGHT AND SHADE LEAVES SAMPLED FROM DIFFERENT HEIGHTS †

	% K		% Mg		% P		% S		p.p.m Zn		p.p.m. Cu	
	Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade
Height 1 (low) *	0.84	1.05	0.26	0.28	0.19	0.20	0.26	0.29	22.3	28.9	13.2	13.9
Height 2 (medium) *	0.90	1.04	0.24	0.27	0.19	0.20	0.26	0.26	20.9	26.6	12.8	14.5
Height 3 (high) *	0.91	0.94	0.25	0.24	0.19	0.19	0.25	0.26	21.0	22.7	13.0	13.8
s.e.	± 0.021		± 0.006		± 0.003		± 0.008		± 0.78		± 0.49	
Min. 5% sig. diff.	0.060		0.018		0.009		0.021		2.20		1.39	

	% N		p.p.m. Mn		p.p.m. B		% Ca		p.p.m. Fe		p.p.m. Mo	
	Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade	Light	Shade
Height 1 (low) *	3.70	3.59	39.5	33.1	35.1	29.1	0.55	0.54	92	91	0.18	0.28
Height 2 (medium) *	3.70	3.53	38.0	30.3	42.5	27.7	0.50	0.48	98	90	0.17	0.26
Height 3 (high) *	3.63	3.58	37.3	34.2	39.4	37.2	0.54	0.53	94	95	0.28	0.17
s.e.	± 0.039		± 2.07		± 1.87		± 0.033		± 2.4		± 0.039	
Min. 5% sig. diff.	0.111		5.85		5.29		N.S.		6.8		N.S.	

† Results expressed on a dry matter basis.

(*Table 1*). It was found that the spur leaves (shade) contained significantly higher contents of phosphorus, potassium, magnesium, copper and sulphur, but lower contents of calcium and iron, than did the leaves collected from the top of the canopy (height 3).

There were strong indications of a gradation in nutrient composition between the lowest leaves (spur leaves (shade), and height 1) and the highest leaves, although the differences between the composition of leaves at two successive heights did not always attain significance. A reduction in the leaf content of phosphorus, potassium, magnesium, copper, sulphur and nitrogen, with height and an increase in the leaf content of calcium, iron and manganese with height was observed.

Sampling Trial II

1. *Variations between light and shade leaves.* In *Table 2* the mean values of the light and the shade leaves have been calculated, and it can be seen that the light leaves contained significantly lower leaf potassium, magnesium, copper, sulphur, and zinc contents, and significantly higher nitrogen, manganese and boron contents than the shade leaves. No significant differences in the nutrient composition between light and shade leaves were observed for phosphorus, iron, calcium and molybdenum, although there was an indication that the phosphorus content of light leaves was lower than that of shade leaves.

2. *Variations due to aspect.* Apart from the differences recorded above between the light (north and south aspects) and shade leaves (east and west aspects), little difference in nutrient composition could be attributed to aspect. The nitrogen content of leaves from the north aspect was significantly, but not markedly, greater than that of leaves from the south aspect (*Table 2*). This difference was largely due to the differences observed at height 2, the nitrogen content of leaves collected from height 1 and height 2 not differing significantly with aspect. The boron content of leaves from the south aspect was significantly greater than that of leaves from the north aspect.

3. *Variations due to height of sampling.* Owing to the differences observed in the nutrient composition of light and shade leaves, the variations in the nutrient composition with height of light and shade leaves have been considered separately (*Table 3*).

(a) *Light leaves.* The leaf potassium content increased significantly, and the leaf magnesium content decreased significantly with greater height of sampling; both these effects were however small. A tendency for leaf boron to increase with height of sampling was also observed. No significant differences in the light leaves due to height were observed with the other nutrients.

(b) *Shade leaves.* The leaf potassium, magnesium, phosphorus, sulphur and zinc content decreased, and the leaf boron content increased significantly with greater height of sampling. These differences with height were greater than those observed in the light leaves. The leaf potassium, magnesium, sulphur and zinc contents were observed to be higher in the shade leaves than in the light leaves (*Table 2*) and it will later be shown with confidence that the leaf phosphorus content follows the same pattern, whilst the boron content is higher in the light leaves. It is therefore probable that the gradual reduction in the leaf potassium, magnesium, phosphorus, sulphur and zinc content, and the increase in leaf boron, with height, observed in the shade leaves, and particularly with regard to the content of leaves collected from height 3, is attributable to the reduced degree of shading of leaves inside the canopy with greater height from the ground. It could be expected that such an effect would be most marked for leaves collected near the top of the tree. No significant difference between the leaf nutrient content of light and shade leaves from height 3 was observed for any nutrient, whilst there were significant differences between the potassium, magnesium, phosphorus, manganese, boron, copper and zinc contents of light leaves from height 3

TABLE 4. TRIAL II. THE EFFECT OF AMMONIUM SULPHATE AND POTASSIUM CHLORIDE ON THE NITROGEN, POTASSIUM, MAGNESIUM, MANGANESE, SULPHUR AND BORON CONCENTRATION OF LIGHT AND SHADE LEAVES †

(The treatment contents expressed as a percentage of the control are given in parenthesis)

	Light	Shade	Light	Shade	Light	Shade
	% N		% K		% Mg	
Control	3.65 (100)	3.52 (100)	0.86 (100)	0.94 (100)	0.27 (100)	0.28 (100)
Potassium chloride	3.56 (97.5)	3.39 (96.3)	1.13 (131.4)	1.33 (141.5)	0.24 (88.9)	0.26 (92.8)
Ammonium sulphate	3.83 (104.9)	3.79 (107.7)	0.67 (77.9)	0.76 (80.8)	0.25 (92.6)	0.25 (89.3)
s.e.	± 0.039		± 0.021		± 0.006	
Min. 5% sig. diff.	0.111		0.060		0.018	
	p.p.m. Mn		% S		p.p.m. B	
Control	32 (100)	28 (100)	0.26 (100)	0.29 (100)	42 (100)	33 (100)
Potassium chloride	31 (96.9)	26 (92.9)	0.25 (96.2)	0.26 (89.6)	42 (100)	33 (100)
Ammonium sulphate	52 (162.5)	44 (157.1)	0.26 (100)	0.26 (89.6)	33 (78.6)	28 (84.8)
s.e.	± 2.07		± 0.008		± 1.9	
Min. 5% sig. diff.	5.8		0.021		5.3	

† Results expressed on a dry matter basis.

and shade leaves from height 2. This again suggests a close similarity between the light leaves and the partially shaded leaves from the top of the canopy, but a dissimilarity between light leaves and heavily shaded leaves. No significant differences in leaf composition of shade leaves due to height were observed with nitrogen, calcium, sulphur, iron and molybdenum.

4. *Observations on the relative sensitivity of leaves from different positions in reflecting changes in tree nutrient content caused by fertiliser applications.* Leaf analysis of mature light leaves from the top of the canopy in the manuring experiment, in which Trial II was undertaken, had consistently and significantly shown that ammonium sulphate increased the nitrogen content and decreased the potassium content, and that potassium chloride increased the potassium content and decreased the magnesium content (SHORROCKS, 1961). There were indications of a positive effect of ammonium sulphate on the manganese content, although a significant effect was not observed on all sampling occasions.

Consequently in the present investigation particular consideration was given to the effects of ammonium sulphate and potassium chloride on the leaf content of nitrogen, potassium, magnesium and manganese, in order to determine whether there were any differences in response to the fertilisers between the various leaf positions.

(a) *Light and shade leaves.* The effects of the fertilisers on the nutrient composition of light and shade leaves are shown in *Table 4*. The significant positive effects of ammonium sulphate on the nitrogen and manganese content, and the significant negative effect on the potassium content were equally clearly observed in both light and shade leaves. The significant negative effect of ammonium sulphate on the magnesium content was observed in the shade leaves only, and only an indication of the same effect was apparent in the light leaves.

A significant positive effect of potassium chloride on the potassium content was observed in both the light and shade leaves, being more marked in the shade leaves. The significant depression of magnesium content caused by potassium chloride was however shown more clearly in the light leaves, though the difference between the two was only slight.

A significant negative effect of ammonium sulphate on the boron content, which was shown by both light and shade leaves, was more marked in the light leaves, whilst the significant negative effects of both ammonium sulphate and potassium chloride on sulphur content was only observed in the shade leaves (*Table 4*).

It is noted that even where the light leaves showed the response more clearly, the shade leaves also showed a significant response of the same nature, whilst there were instances where a response was only evident in the shade leaves. No significant effects of fertiliser on the leaf content of the phosphorus, calcium, zinc, copper, iron, and molybdenum were observed.

(b) *Leaves from different heights.* The effects of fertiliser on the nitrogen, potassium, magnesium, manganese, sulphur and boron content of leaves from different heights in both the light and shade are shown in *Table 5*. It can be seen that the lowest shade leaves showed most clearly the effects of ammonium sulphate on the leaf nitrogen and sulphur contents, and also the effect of potassium chloride on the leaf potassium and sulphur contents. Although there was little difference between the effect of ammonium sulphate on the nitrogen content of light and shade leaves when bulked, it would appear that the lower shade leaves are more sensitive to change. The significant negative effect of ammonium sulphate on potassium content was shown equally clearly at all leaf positions. Light leaves from near the top of the tree showed the significant negative effect of potassium chloride on the magnesium content, and the significant positive effect of ammonium sulphate on manganese content, more clearly than the other leaves. The negative effect of ammonium sulphate on boron content was shown almost equally clearly by the lowest

TABLE 5. TRIAL II. THE EFFECT OF AMMONIUM SULPHATE AND POTASSIUM CHLORIDE ON THE NITROGEN, POTASSIUM, MAGNESIUM, MANGANESE, SULPHUR AND BORON CONCENTRATION OF LEAVES FROM DIFFERENT HEIGHTS IN THE LIGHT AND SHADE †

(The values in bold figures indicate the maximum responses to the fertilisers)

	Light			Shade			Light			Shade		
Height	1 Low	2 Medium	3 High	1 Low	2 Medium	3 High	1 Low	2 Medium	3 High	1 Low	2 Medium	3 High
			% N						% K			
Control	3.68	3.64	3.62	3.46	3.58	3.52	0.78	0.88	0.88	0.95	0.98	0.88
Potassium chloride	3.54	3.56	3.58	3.39	3.35	3.42	1.10	1.12	1.18	**1.44**	1.32	1.21
Ammonium sulphate	3.89	3.90	3.70	**3.92**	3.66	3.80	0.65	**0.69**	**0.68**	**0.76**	0.80	0.72
	± 0.068, Min. 5% sig. diff. 0.192						± 0.037, Min. 5% sig. diff. 0.104					
			% Mg						p.p.m. Mn			
Control	0.28	0.25	0.28	0.30	0.28	0.27	42	27	29	29	26	28
Potassium chloride	0.25	0.23	**0.22**	0.28	0.26	0.23	29	33	30	24	26	27
Ammonium sulphate	0.26	0.24	0.26	0.28	0.26	**0.22**	48	**54**	**52**	46	38	48
	± 0.011, Min. 5% sig. diff. 0.032						± 3.6, Min. 5% sig. diff. 10.1					
			% S						p.p.m. B			
Control	0.28	0.25	0.26	0.32	0.28	0.27	41	42	44	28	29	42
Potassium chloride	0.26	0.26	0.24	**0.27**	0.25	0.26	36	50	39	31	31	37
Ammonium sulphate	0.26	0.26	0.25	**0.28**	0.26	0.24	**29**	35	36	28	23	**32**
	± 0.013, Min. 5% sig. diff. 0.037						± 3.2, Min. 5% sig. diff. 9.2					

† Results expressed on a dry matter basis.

light leaves and by the highest shade leaves, whilst the negative effect on the magnesium content was only observed in the highest shade leaves.

DISCUSSION

The results of Trial I indicated little difference in the nutrient composition of light and shade leaves. In view of the positive results of Trial II, in which it was shown that the shade leaves contained higher potassium, magnesium, sulphur, copper and zinc contents, and lower nitrogen, manganese and boron contents than did the light leaves, it is considered that the difference in the position of sampling of the shade leaves in the two investigations could account for the different results. The shade leaves in Trial I were less shaded than in Trial II owing to their situation on the inter-row side of the tree and not in the intra-row side as in Trial II.

The results of Trial I indicated differences in leaf nutrient composition with height from the ground, and particularly between the lowest and highest leaves, with a gradation between these two extremes. In Trial II it was possible to differentiate between the light and shade leaves with regard to the variation in nutrient composition with height. Differences in nutrient composition with height from the ground were clearly demonstrated for the shade leaves only, their leaf potassium, magnesium, phosphorus, sulphur and zinc contents decreasing with height, and the leaf boron content increasing with height. These results confirm those observed in Trial I between the contents of lowest leaves and highest leaves (light and shade leaves considered together).

The differences in shade leaf composition can be attributed to the reduced degree of shading with height, and the differences are compatible with those observed between light and shade leaves.

Only slight differences in the leaf potassium and magnesium contents of light leaves collected from different heights were observed, and in no case was a significant difference between the composition of light leaves from heights 2 and 3 observed. No significant differences between the composition of light and shade leaves collected from the top of the tree were observed and little difference could be attributed to aspect with either the light or shade leaves.

It is concluded that there is little difference in leaf nutrient composition of mature leaves exposed to full sunlight, and that the collection of such leaves would involve a very small sampling error. The collection of shaded leaves from within any part of the canopy would result in the introduction of a larger sampling error due particularly to the varied degree of shading of the leaves in different positions.

Little difference between the nutrient composition of the shaded lower leaves and the spur leaves (shade) were observed and it is possible that the collection of a mixed sample of spur leaves (shade) and low shade leaves would introduce only a small amount of variability. It is considered that the sampling of such particular shade leaves is worthy of further research. This alternative is envisaged owing to the practical difficulties encountered in sampling leaves from the top of the tree, and because of the scarcity of spur leaves (shade). There would appear then to be four practical, alternative leaf sampling techniques; firstly the collection of leaves exposed to full sunlight, secondly the collection of spur leaves (shade), thirdly the collection of low shaded leaves and fourthly the collection of a mixture of the two latter types of leaves.

The preliminary results on the effects of ammonium sulphate and potassium chloride on leaf nutrient composition at the different positions, indicated that in general shade leaves reflected the effects of fertiliser more clearly than light leaves. In cases where the light leaves were superior the difference was only slight. The only exceptions were in the effects of ammonium sulphate on the manganese content and of potassium chloride on the magnesium content. On the basis that those leaves which are more sensitive to changes in nutrient composition imposed by treatment are likely to be the leaves that reflect more clearly the current nutrient status

of the tree, these results suggest that, for advisory purposes, shade leaves would be more suitable for sampling than light leaves.

The present data are considered insufficient to warrant detailed comparison between fertiliser responses and nutrient contents of each individual leaf position, and this problem and further findings regarding the sensitivity of different leaf positions to changes in nutrient composition will be dealt with in a later paper. Clonal differences in response may occur, and it is possible that sensitivity to changes, as measured by sampling, may vary with time of year as found by Goodall (1943, 1945) with apples. It is therefore necessary to study this problem by samplings carried out at different times of the year and with different planting materials, before a final assessment can be made.

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Pollination of Hevea in Malaya

B. SRIPATHI RAO

Observations on the insects visiting flowers of Hevea were made in three successive years and their possible role as pollinators noted. Those entering flowers are listed.

Hevea brasiliensis in Malaya produces flowers twice a year—during a main flowering season from February to June, and a subsidiary one from August to October. Though flowers are produced in abundance, the number of fruits produced is small. MAAS (1919) estimated that even in a good flowering season not more than 3 per cent of the female flowers developed into fruit. In a seed garden in Malaya, the percentages of female flowers that later bore mature fruits in the years 1958, 1959 and 1960 were observed to be only 0.3, 1.6 and 0.5 respectively, but higher figures were obtained in 1959 following hand pollination at the Experiment Station of the Rubber Research Institute of Malaya (ROSS, 1961). The degree of success at harvest depended on the time of hand pollination; averaging the results of all crosses, pollinations in the first period give 8.4% mature fruits as compared with 4.6% from later pollinations. Where unselected seed is required for planting, to be budded later, a poor yield of seed is of little consequence because of the large area available for collection. In a seed garden, on the other hand, a low production of seed in the main planting season is a serious matter. The results of hand pollinations suggest that the production of a seed garden might be improved if the degree of natural pollination could be increased. For this reason the mechanism of natural pollination is worthy of study.

Although the size of the pollen grain is not large (about 44 μ in diameter), pollen does not appear to be carried by wind. Inflorescences enclosed in insect-proof bags did not set seed (MAAS, 1919; MORRIS, 1929; MUZIK, 1948). Vaseline-coated slides placed near inflorescences failed to collect pollen (MUZIK, 1948). The author failed to collect any on slides in a Hirst volumetric spore trap that was run for three days within fifty feet of heavily flowering trees on the R.R.I.M. Experiment Station; nor was any detected in centrifuged leaf washings of the upper surface of one hundred leaves taken at random from just below the inflorescences at four different localities.

The structure of the flowers, their colour and fragrance, the stickiness of the pollen grain and of the stigmatic surface and the presence of a nectary, point to insects as being the most likely agents of pollination. In Malaya, MORRIS (1929) recorded many types of insects—bees, flies, bugs, caterpillars, beetles, weevils and ants—as having been seen on or around inflorescences, but he did not produce evidence of their ability to carry pollen from male to female flowers. He had seen bees of at least three species frequently entering male flowers to collect pollen, and a small fly and two minute beetles inside female flowers.

The first methodical study of insect visitors to *Hevea* flowers, and the first demonstration that they effect pollination, were made by WARMKE (1951) in Puerto Rico. By placing a sticky material on the tips of petals and by means of adhesive cards wrapped round the inflorescences in loose cylinders he caught a large number of insects, mostly thrips and small flies. Of the latter a majority were Ceratopogonid midges of the genera *Dasyhelea, Atrichopogon* and *Forcipomyia,* whose antennal and body hairs were found sticking on the stigmas. Continuing his observations in Brazil, the same author (WARMKE, 1952)

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again found abundant Ceratopogonid midges visiting male and female flowers for about 1 to 1½ hours after sunrise and for the same period before sunset. He had no doubt that they were largely responsible for pollination.

Observations in Malaya

Inflorescences with freshly opened flowers were kept under observation during the months of March and April 1953, April 1954 and April, May and June 1955 on the R.R.I.M. Experiment Station. In 1953, low flowering branches were chosen for convenience of observation, but the collection was poor, heavy infection of the inflorescences with *Oidium heveae* resulting in premature flower fall. In the following years a ladder or tower was used, bringing the observer within reach of inflorescences at a height of 25 feet. Observations were made at different times of the day, but mostly during the two hours after sunrise and two hours before sunset, for about 26 hours in 1953, 18 hours in 1954 and 21 hours in 1955. A considerable collection of insects was made, even though it involved waiting for a long time, without disturbing the flowers, before an insect could be seen to approach.

To trap the insects, the following method was used, based on that of Posnette (1944) who collected insects from cocoa flowers in Trinidad. When an insect was seen to enter a flower, a 7 × 1 in. specimen tube was lowered over it, the cork being used to prevent the insect's escape. The flower was released after the insect had come out into the tube.

Most of the insect visitors to the flowers were thrips, midges of the families Ceratopogonidae, Chironomidae and Cecidomyiidae, or Scatopsid flies, but no group could be said to be numerous. The thrips could be collected in the mornings and evenings; the midges mainly in the evenings. Small numbers of other flies—Drosophilidae, Calliphoridae, Psychodidae, Sciaridae, Phoridae, Muscidae and Chloropidae—were also collected. Parasitic Hymenoptera of the families Braconidae, Encyrtidae, Eulophidae, Ceraphronidae and Cynipidae also visited the flowers. It is possible that many of them are parasites of Lymantriid (*Euproctis subnotata* Walk.), Noctuid (*Oruza vacillans* Walk.) and Geometrid (*Hemithea costipunctata* Moore and *Chloroclystis sp.*) caterpillars frequently seen feeding on the flowers and tender leaves. Bees (*Trigona spp.*), ants (*Tapinoma spp.*), Mirid bugs (*Lygus sp.*) and the fungivorous beetles *Thea bistigmosa* (Muls) (*Coccinellidae*) and *Anisomeristes sp.* (*Corylophidae*) were frequently found on the inflorescences. Strong wind, cloudy weather and rain reduced the number of visitors. The Diptera, Thysanoptera and the parasitic Hymenoptera which visited the flowers are listed in *Table 1.*

Thrips were the commonest insects in the flowers, moving in and out of them, or getting stuck to the black spots of coagulated latex often found on the petals, believed to result from insect injury. Four species — *Taeniothrips* (*Rhopalandrothrips*) *minor* (Bagnall), *Thrips hawaiiensis* (Morgan) and *Thrips florum* (Schmutz)—comprised most of the thrips visitors. Many had pollen sticking to their wings.

The Ceratopogonidae were the most numerous of the midges that visited the flowers. In the 1953 collection the family was represented by a few specimens of *Forcipomyia* sp. collected only from male flowers, but in 1954 a good collection of individuals of this genus and of *Dasyhelea* sp. was made from both male and female flowers. In the 1955 collection, the Ceratopogonidae and Scatopsidae (mostly unidentified) were almost equally numerous. Altogether, over the three years, Ceratopogonidae were seen to enter male or female flowers thirty-two times, the Scatopsidae fourteen times, Cecidomyiidae twelve times and Chironomidae six times.

The midges generally fly round the inflorescence for a while before alighting on an open flower; after some hesitation they move down into the corolla tube with their dorsal side towards the stigma or anthers. They move around the ovary or staminal column, stopping frequently, apparently to feed. They

TABLE 1. INSECT VISITORS TO HEVEA FLOWERS ON THE RUBBER RESEARCH INSTITUTE EXPERIMENT STATION DURING 1953, 1954 AND 1955

Order and family	Identification	Number of times observed	Remarks
DIPTERA:			
Calliphoridae	*Idiella divisa* Walk.	2	Insert mouth parts into open flowers.
Cecidomyiidae	Genus undetermined	12	Enter male and female flowers; pollen on body, legs and antennae.
Ceratopogonidae	*Dasyhelea sp.*	11	Enter male and female flowers; pollen on body, legs and antennae.
	Forcipomyia spp.	16	Enter male and female flowers; pollen on body, legs and antennae.
	Genus undetermined	5	Enter male and female flowers; pollen on body, legs and antennae.
Chironomidae	*Metriocnemus sp.*	2	One entering male and the other entering female flower.
	Orthocladius sp.	2	One inside male flower and the other stuck to stigma of female flower.
	Smittia sp.	2	Both inside female flowers; pollen on antennae.
Chloropidae	*Oscinella sp.*	3	Two entering male flowers and one entering female flower; pollen on head and wings.
Drosophilidae	*Drosophila sp.*	4	Enter male and female flowers.
Ephydridae	*Hecamedoides sp.*	1	Enters male flower.
Muscidae	*Atherigona sp.*	1	Enters male flower.
Phoridae	*Megaselia sp.*	3	Enter male flowers.
Psychodidae	*Psychoda sp.*	2	Enter female flowers, pollen on mouth parts.
Scatopsidae	*Scatopse sp.*	2	Enter male flowers.
	Genus undetermined	12	Enter male and female flowers; some with pollen on body and wings.
Sciardae	*Bradysia sp.*	1	Enters male flower.
Unidentified Nematocera		6	Caught within male and female flowers; some with pollen on antennae.
HYMENOPTERA:			
Braconidae	*Apanteles hemitheae* Wilkn.	1	Inside female flower.
Ceraphronidae	*Aphanogmus sp.*	2	Inside male flowers.
Cynipidae	*Chrestosema sp.*	1	Inside male flower.
	Ganaspis sp.	1	Stuck to petal of female flower.
	Nedinoptera sp.	1	Inside male flower.
	Pseudeucoila sp.	2	One inside male and the other inside female flower.
	Rhoptromeris sp.	2	Inside male flowers.
	Trybliographa sp.	2	Stuck to petal of male flowers.
Encyrtidae	Genus undetermined	1	Inside female flower.
Eulophidae	Genus undetermined	3	Inside male and female flowers.
	Syntomosphyrum obscuriceps Ferr.	1	Inside male flower.
	Tetrastichus sp.	1	Inside female flower.
THYSANOPTERA:			
Thripidae	*Taeniothrips* (*Rhopalandrothrips*) *minor* Bagnall	many	Inside male and female flowers and stuck to latex on petals.
	Taeniothrips (*Lefroyothrips*) sp.	2	One inside female flower and the other stuck to petal of male flower.
	Taeniothrips spp.	2	Inside female flower and stuck to male flower.
	Thrips hawaiiensis Morgan	many	Inside male and female flowers and stuck to latex on petals.
	Thrips florum Schmutz	many	Inside male and female flowers and stuck to latex on petals.
	Thrips pallipes Moulton	1	Inside male flower.
	Thrips spp.	2	Inside male and female flowers.

Figure 1. A dead midge (Orthocladius sp.) stuck to the stigma and petals of female flower.

may spend anything from two to five minutes within the flower. Many emerged with pollen sticking to them—particularly those that have brush-like antennae. Dead insects were sometimes seen stuck to the petals or stigma (*Figure 1*).

In 1955, one hundred and fifty female flowers that had opened the previous day were collected from seven different localities and their stigmas examined: insect hairs, apparently the antennal and body hairs of the midges, were seen on the stigmas of 8 out of 20, 5 out of 15, 5 out of 12, 2 out of 12, 7 out of 50, 3 out of 18 and nil out of 23 flowers. (*Figure 2*). Bunches of short bristles resembling those on the wings of thrips were also occasionally noticed on the stigmas.

DISCUSSION

Only the midges, thrips and the parasitic Hymenoptera, which have been seen to visit both male and female flowers, need to be considered as possible pollinators. The other insects merely rest on the inflorescence or on unopened flowers, feed on the flowers or collect pollen.

The Ceratopogonidae, and to a lesser extent the Chironomidae, Cecidomyiidae and Scatopsidae, emerge as the most important pollinating agents. Their minute size and clothing of long hairs, and their capacity for sustained flight, make them ideally suited to this function. They have been seen to visit both male and female flowers, and often to have pollen attached to them.

The thrips, though more numerous, can be expected to play a smaller role, as they are not active fliers. However, they may transfer pollen between flowers on neighbouring inflorescences, or on neighbouring trees. The role of parasitic Hymenoptera is uncertain. They are much less hairy; further, their presence is dependent upon the presence of their hosts.

CONCLUSIONS

Warmke's observations on the role of Ceratopogonid midges in the pollination of *Hevea* in Puerto Rico and Brazil can be said to apply broadly in Malaya. But, whereas in Brazil

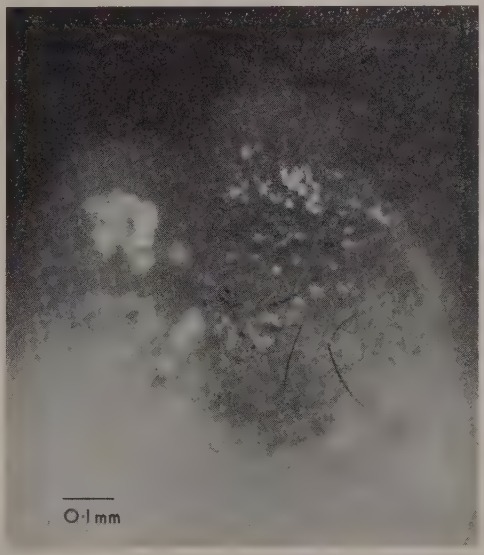

Figure 2. Insect hairs adhering to the surface of one lobe of the stigma.

they were 'so numerous that half a dozen may be observed at one time around a single inflorescence', in Malaya their visits are infrequent.

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Manganese Deficiency in Hevea: The Effect of Soil Application of Manganese Sulphate on the Manganese Status of the Tree

V. M. SHORROCKS and G. A. WATSON

The effect of soil application of manganese sulphate on the nutrient status of Hevea brasiliensis trees affected by a deficiency of manganese in the soil was investigated. Significant increases in the manganese content of leaf, latex, bark and bark scrap rubber were observed, with increases more clearly shown in leaves and bark than in the bark scrap rubber and latex. Different rates and frequencies of application of manganese sulphate were used, and it was concluded that two broadcast applications of four ounces per tree was the minimum treatment required to improve the manganese status of trees affected by moderate to severe manganese deficiency.

Many cases of manganese deficiency on immature and mature *Hevea* have been observed throughout Malaya (RUBBER RESEARCH INSTITUTE OF MALAYA, 1961a) since the description of the characteristic symptoms of deficiency by BOLLE-JONES (1956). The deficiencies are evidently the result of absolute deficiencies of manganese in the soil, since *Hevea* is grown on soils of low organic matter content with pH values in the range 4.5 – 5.5 — conditions under which soil manganese is freely available, if present. This is in contrast with the more usual cases of induced manganese deficiency where soil manganese is rendered unavailable for plant uptake because of high pH or organic matter levels (MULDER AND GERRETSON, 1952).

AKHURST (1933) found that the total manganese content of Malayan soils (except some derived from limestone) was low, averaging 279 p.p.m. This low level of manganese in many Malayan soils is probably the direct result of the severe leaching of the acid soils under conditions of high rainfall. The exchangeable manganese content of typical inland Malayan soils is normally less than 0.036 m-equiv./100 g while levels of less than 0.0071 m-equiv./100 g are frequently found. These figures can be compared with levels of 0.18 – 0.72 m-equiv./100 g reported by WATSON (1960) in some temperate climate soils where manganese uptake was excessive at pH levels of about 4.5. These can only be approximate comparisons of course, for other factors such as the exchangeable calcium, soil aeration and the proportion of total and 'reducible' manganese present will also affect the availability of manganese, but these figures represent the general order of differences that are found.

Since a deficiency of manganese can adversely affect tree growth (BOLLE-JONES, 1957) it is important to determine ways in which the condition can be remedied. This paper presents results obtained from two experiments laid down primarily to study manganese uptake by *Hevea*, and also results from three other experiments which involve treatments with manganese sulphate. In all these experiments soil applications of manganese sulphate fertiliser have been tested, rather than application by spray or injection, since the problem is one of simple soil deficiency and not of deficiency induced by unfavourable soil conditions.

The effects on the manganese status of the tree have been followed by periodical leaf, latex, bark and bark scrap rubber sampling. The study of bark scrap rubber was required because a high concentration of manganese

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in the rubber has the deleterious effect of promoting oxidation, and there is a legal tolerance limit of 10 p.p.m. on the amount of manganese that may be present in commercial rubber (RUBBER MANUFACTURERS ASSOCIATION N.Y., 1957).

The studies were carried out on areas showing leaf symptoms of manganese deficiency; otherwise the areas were not markedly deficient in any particular nutrient, but were situated on soils on which responses to normal NPK fertilisers could be expected.

SAMPLING METHODS

Details of the five fertiliser experiments are given in *Table 1*. Sampling details are given below, and a discussion of each experiment and its results follow.

Leaf Sampling

The same type of leaf was sampled in all experiments. Two mature whorls exposed to full sunlight and situated on the outside of the canopy were chosen on each tree sampled, and the four basal leaves on both whorls were collected.

In Experiments 1 and 2, six trees were selected at random in each plot at the start of the investigation and were sampled throughout.

In Experiment 3, sixteen trees per plot were selected at random, and in Experiments 4 and 5 six trees per plot were selected. All leaves collected within each plot were bulked for analysis.

Latex Sampling

In Experiment 1, latex sampling was carried out at approximately the same date as leaf sampling, and from the same trees. An aliquot of the total latex from the six trees, collected after latex had stopped flowing, was taken for analysis.

Bark Sampling

In Experiment 1, bark samples were taken from a height of 100–106 inches above the union from all recorded trees in each plot.

Bark Scrap Rubber Sampling

In Experiments 1 and 2, bark scrap rubber samples were collected from all recorded trees twenty-four hours after tapping, and the samples from all plots of the same treatment were bulked for analysis. The samples were not washed prior to analysis, although contaminating particles of bark were removed by hand.

The preparation and analysis of the leaf and bark samples were carried out by the methods described by BOLLE-JONES (1954)

TABLE 1. DETAILS OF EXPERIMENTS

Experiment No.	Clone	Year budded	Plot size (acres)	Rate of manganese sulphate application (oz/tree/annum)	Dates of application
1	Tjir 1, BR 2 PB 49, AVROS 157	1950	0.11	0, 4 and 8*	First, 8.5.57; second, 27.5.58
2	RRIM 509	1950	0.11	0 and 4	First, 11.6.58; second, 9.5.59; third, 23.4.60
3	Tjir 1 Clonal Seedlings	1955**	0.50	0 and 2	First, 23.9.59
4	PB 86	1950	0.54	0 and 4	First, 15.2.60
5	PB 86	1950	0.73	0 and 4	First, 19.2.60

* Only half the plots received the second application.

** Year of planting.

and by Bolle-Jones, Mallikarjuneswara and Ratnasingam (1957). The petioles and mid-ribs of leaves were discarded before analysis.

Soil Sampling

In Experiment 1 soil samples were collected once, approximately four years after the first experimental manuring. Eight core samples, 0–12 in., were taken from the intra-row areas in each plot. The total soil manganese content was estimated after acid extraction by the method described by Piper (1950), and the exchangeable soil manganese after extraction with *N* ammonium acetate.

EXPERIMENT 1

This experiment was located in an avenue planting sited on a coarse sandy, granite-derived soil cleared from jungle in 1941, cultivated for food crops and then planted as a seed garden in 1949, with five *Hevea* clones Tjir 1, BR 2, PB 49, AVROS 157 and RRIM 509. The planting distance was 60 × 4½ ft. Along each of three adjacent rows, six single-row plots of twenty trees, each containing similar numbers of the different clones, were marked out, the two trees at each end of the plots being used as 'guard' trees. To minimise poaching across the rows, a plough furrow was driven up the inter-row areas in the first year, but this was not repeated after analytical results had revealed few, if any, poaching effects.

Pre-treatment sampling of leaves and bark was carried out, before application of manganese sulphate (30% manganese content) at rates of 0, 4 oz and 8 oz per tree. Treatments were in sextuplicate, and the fertiliser was sprinkled evenly over a 10 ft wide strip on both sides of the tree rows.

Analytical results in the first year of the experiment showed a marked effect of the treatments on the manganese content of the trees. In order to test the persistence of this effect only half of the plots initially manured received manganese sulphate in the second year.

Leaf, latex, bark and bark scrap rubber samples were taken at intervals as indicated in *Tables 3*, *4* and *5*, and *Figures 1* and *2*.

RESULTS OF EXPERIMENT 1

Visual Observations

Visual assessment in the field of the incidence of manganese deficiency symptoms indicated an improvement of the foliage at five months after the first application of manganese sulphate. An assessment made after thirty months indicated that the order of effectiveness of the treatments in improving foliage condition was:

2×8 oz $> 2 \times 4$ oz $> 1 \times 8$ oz $> 1 \times 4$ oz

Visual assessment of the severity of the symptoms shown by individual plot samples of 48 leaves each was carried out on twelve sampling occasions, and the comparison of the assessment with the leaf manganese contents is given in *Table 2*.

TABLE 2. EXPERIMENT 1. MANGANESE CONCENTRATION IN MANGANESE DEFICIENT LEAVES

Symptom expression	No. of cases	Mn p.p.m. mean	Standard deviation
None	134	88	44.9
Slight	48	51	22.6
Moderate	15	23	11.0
Severe	19	19	5.2

Results indicate that a sample that exhibits definite symptoms of manganese deficiency will have a content of less than 50 p.p.m. manganese, while severe symptoms will be associated with a content of approximately 20 p.p.m. manganese.

Leaf Analysis

One application. The first post-treatment sampling, at six months after application, showed that both the 4 oz and the 8 oz applications had significantly increased leaf manganese content (*Table 3*). This effect reached its maximum at twelve months after applica-

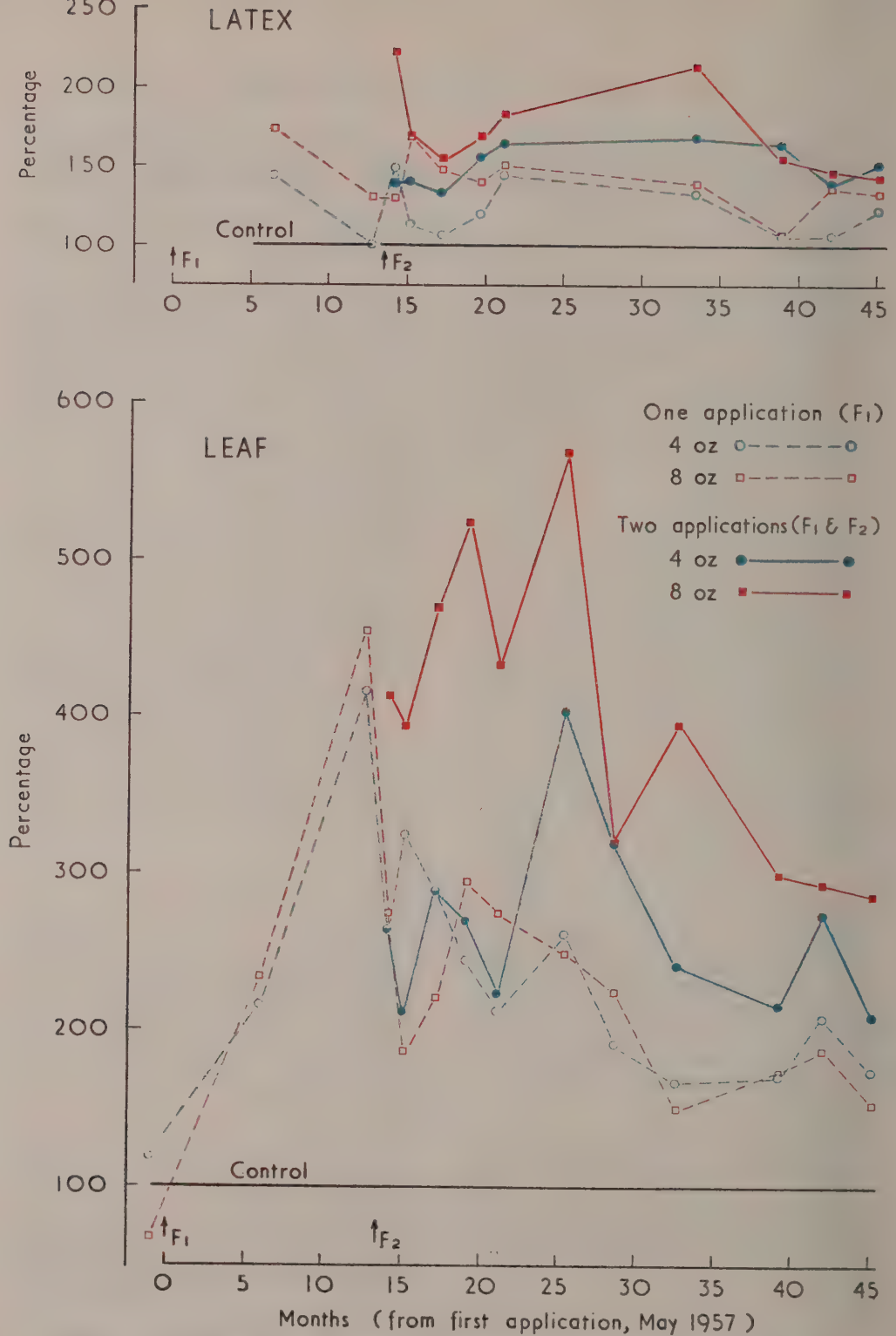

Figure 1. Experiment 1. The effect of manganese sulphate on the leaf and latex manganese content. (Manganese contents are expressed as a percentage of control for each sampling occasion).

tion when the leaf manganese content was increased to a level four and a half times greater than that of the control (*Figure 1*). The significant positive effect was observed for a period of thirty-six months after application, with no significant difference between the 4 oz and 8 oz applications becoming evident.

Two applications. The leaf manganese content was increased further by the second application of manganese sulphate, with a maximum mean of 159 p.p.m. observed after two applications of 8 oz (*Table 3*). The two applications of 8 oz caused marked significant increases in the leaf manganese content during the period studied, which covered thirty months after the second application. The increases reached a maximum at eleven months, when the manganese content was five and a half times greater than that of the control (*Figure 1*).

Whilst the two applications of 4 oz did not always result in a significant increase in the leaf manganese content, there were strong indications of a positive effect throughout. Likewise there were indications that the effect of the two 8 oz applications was greater than that of the two 4 oz applications. The decline in the leaf manganese content following the two applications was less evident than that following the one application, and the lowest manganese content recorded following the double 8 oz applications was 82 p.p.m.

The order of effectiveness of manganese sulphate in improving the leaf manganese status, judged from the data covering the third year after the first application, was as follows:

2×8 oz $> 2 \times 4$ oz $> 1 \times 8$ oz $= 1 \times 4$ oz

No consistent significant effects of manganese sulphate on the magnesium, potassium, calcium, nitrogen and phosphorus contents of the leaves were observed (RUBBER RESEARCH INSTITUTE OF MALAYA, 1961b). There were however indications of a negative effect of manganese sulphate on the leaf magnesium and potassium contents, and a positive effect on the leaf calcium content.

Latex Analysis

One application. The latex manganese content was significantly increased by the 8 oz, but not by the 4 oz application (*Table 4*). A significant, positive, residual effect of the 8 oz application was observed on two sampling occasions in the second year after application, and there were indications of a similar residual effect on other occasions. The maximum mean concentration, observed at twelve, fourteen and thirty-one months after the single application of 8 oz, was 2.4 p.p.m. manganese.

Two applications. Both the two 4 oz and the two 8 oz applications caused significant increases in the latex manganese measured at two months after the second application (*Table 4*), and these effects were maintained for thirty months after the second application.

On three sampling occasions the positive effect of the two 8 oz applications was significantly greater than that of the two 4 oz applications: there were also strong indications of such a difference persisting for up to nineteen months after the second application. The maximum mean concentration, observed nineteen months after the second 8 oz application, was 3.7 p.p.m. manganese, approximately two and a quarter times greater than the manganese content of the control (*Figure 1*).

The order of effectiveness of manganese sulphate in increasing the latex manganese status, was as follows (*Figure 1*):

2×8 oz $> 2 \times 4$ oz $> 1 \times 8$ oz $= 1 \times 4$ oz

No consistent significant effects of manganese sulphate on the nitrogen, phosphorus, potassium and copper content of the latex were observed (RUBBER RESEARCH INSTITUTE OF MALAYA, 1961b). There were however indications of a positive effect of manganese sulphate on the latex magnesium content.

Bark Analysis

A marked positive effect on the bark manganese content of both the 4 oz and the 8 oz dressings was recorded after six months (*Table 5*). Eight months after the second application it was observed that the bark

TABLE 3. EXPERIMENT 1. THE EFFECT OF MANGANESE SULPHATE ON THE MANGANESE CONCENTRATION IN THE LEAVES

Dates of Application: first (to all plots), 8.5.57 (F1); second (to half of the plots), 27.5.58 (F2)

	p.p.m. Mn in dry lamina													
Dates of sampling	17.4.57 (F1)	31.10.57	21.5.58 (F2)	16.6.58	16.7.58	8.9.58	3.11.58	29.12.58	27.4.59	5.8.59	23.11.59	6.5.60	8.8.60	4.11.60
Control	32	29	29	49	38	30	25	28	26	36	45	61	41	32
Mn_1 4 oz 1957 only	38	63	121	99	107	78	53	48	63	54	62	92	67	50
Mn_2 8 oz 1957 only	22	68	132	103	62	60	64	62	60	63	56	93	60	44
s.e.	± 11.1	± 8.2	± 21.0	± 11.1	± 9.0	± 10.3	± 8.7	± 4.3	± 4.7	± 2.7	± 9.7	± 7.6	± 8.0	± 4.7
Min. 5% sig. diff.	N.S.	25.9	66.3	43.5	35.5	40.5	34.1	17.0	18.3	10.5	N.S.	29.8	N.S.	N.S.
Control				26	28	24	18	17	22	20	29	45	23	25
Mn_1 4 oz 1957 and 1958				99	70	78	58	50	97	90	90	115	88	60
Mn_2 8 oz 1957 and 1958				156	130	127	113	98	137	90	147	159	95	82
s.e.				± 11.7	± 13.6	± 25.5	± 21.3	± 16.0	± 30.7	± 17.7	± 13.4	± 25.1	± 14.7	± 16.4
Min. 5% sig. diff.				46.1	53.4	100.1	83.4	62.8	120.5	69.6	52.6	98.7	57.8	53.6

TABLE 4. EXPERIMENT 1. THE EFFECT OF MANGANESE SULPHATE ON THE MANGANESE CONCENTRATION IN THE LATEX

Dates of Application: first (to all plots), 8.5.57 (F1); second (to half of the plots), 27.5.58 (F2)

	p.p.m. Mn in total solids										
Dates of sampling (F1)	31.10.57	23.5.58 (F2)	16.6.58	18.7.58	8.9.58	19.11.58	29.12.58	16.12.59	16.5.60	14.8.60	14.11.60
Control	1.3	1.8	1.3	1.6	1.4	1.5	1.5	1.8	1.3	1.3	1.5
Mn_1 4 oz 1957 only	1.9	1.8	1.8	1.6	1.5	1.5	2.2	2.3	1.3	1.3	1.5
Mn_2 8 oz 1957 only	2.3	2.4	1.6	2.4	2.1	2.0	2.3	2.4	1.3	1.7	1.6
s.e.	± 0.26	± 0.22	± 0.31	± 0.18	± 0.16	± 0.21	± 0.29	± 0.28	± 0.06	± 0.28	± 0.21
Min. 5% sig. diff.	0.83	N.S.	N.S.	0.71	0.64	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Control			1.0	1.2	1.3	1.3	1.5	1.6	1.1	1.1	0.8
Mn_1 4 oz 1957 and 1958			1.7	2.0	1.9	2.2	2.5	2.9	2.0	1.7	1.7
Mn_2 8 oz 1957 and 1958			2.7	2.4	2.2	2.4	2.8	3.7	1.9	1.8	1.6
s.e.			± 0.47	± 0.09	± 0.08	± 0.06	± 0.07	± 0.20	± 0.10	± 0.22	± 0.09
Min. 5% sig. diff.			N.S.	0.34	0.33	0.23	0.29	0.78	0.38	N.S.	0.35

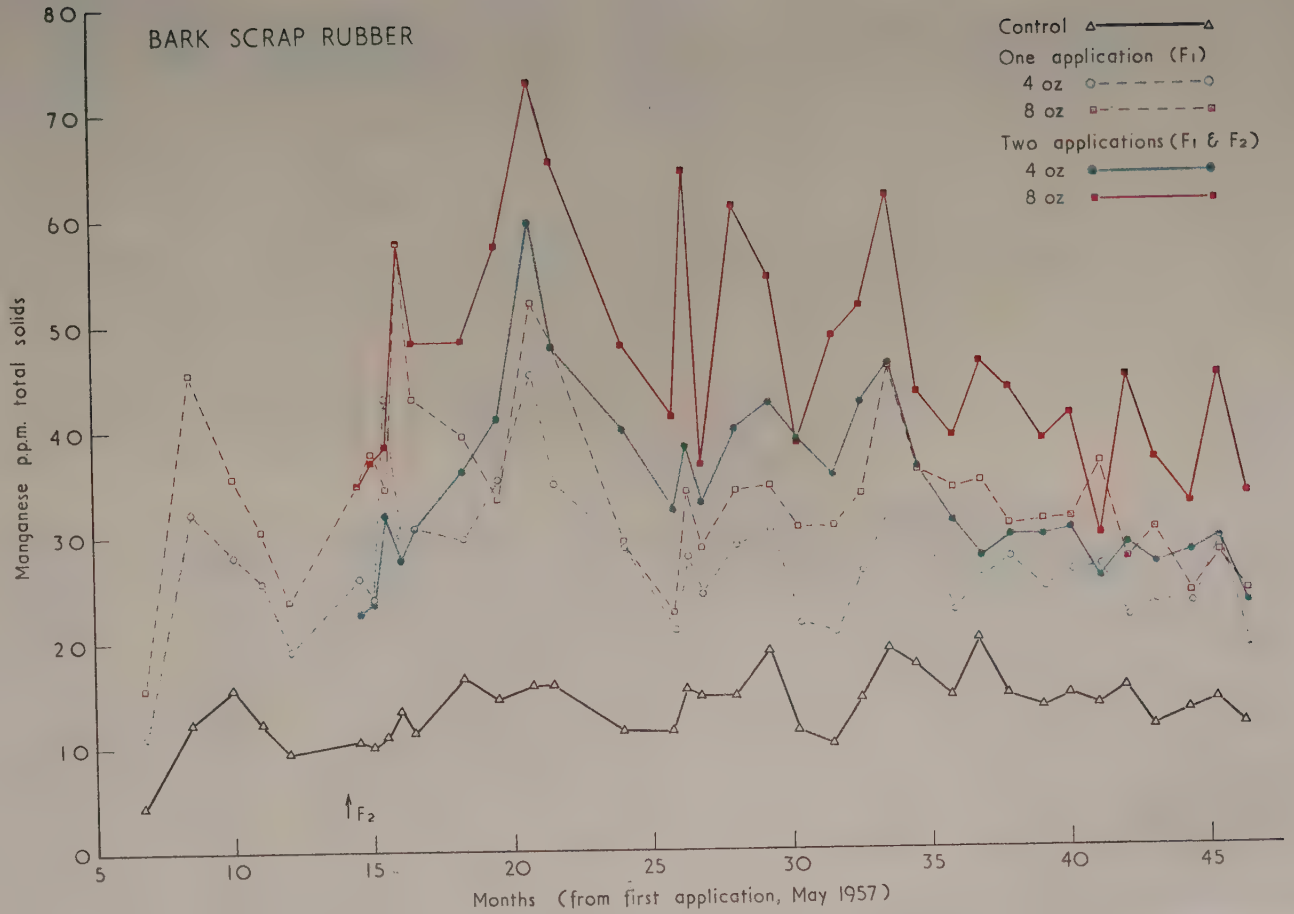

Figure 2. Experiment 1. The effect of manganese sulphate on the manganese content of bark scrap rubber.

TABLE 5. EXPERIMENT 1. THE EFFECT OF MANGANESE SULPHATE ON THE MANGANESE CONCENTRATION IN THE BARK

Dates of Application: first (to all plots), 8.5.57 (F1); second (to half of the plots), 27.5.58 (F2)

	p.p.m. Mn in dry bark			
Dates of sampling	16.4.57 (F1)	31.10.57 (F2)	21.1.59	4.3.61
Control	43 (100)	44 (100)	69	54
Mn_1 4 oz 1957 only	39 (91)	97 (220)	122 (230)*	63 (134)*
Mn_2 8 oz 1957 only	34 (79)	117 (266)	110 (207)*	62 (132)*
s.e.	± 8.6	± 9.7	± 22.5	± 6.5
Min. 5% sig. diff.	N.S.	30.7	N.S.	N.S.
Control			37	40
Mn_1 4 oz 1957 and 1958			147 (277)*	77 (164)*
Mn_2 8 oz 1957 and 1958			224 (422.6)*	92 (196)*
s.e.			± 23.3	± 7.8
Min. 5% sig. diff.			91.3	30.8

The contents expressed as a percentage of the control are given in parenthesis.

* Values calculated using mean of all control plots.

manganese content had been further increased significantly by the two applications of 4 oz and 8 oz, whilst at the same time there were strong indications of a residual effect due to one application of 4 oz or 8 oz. Significant residual effects of the two applications only were observed after four years.

The increases in the bark manganese content were of the same order as the increases in the leaf manganese content for the individual sampling occasions. The order of effectiveness of the different applications of manganese sulphate in increasing the bark manganese content was the same as that observed for the leaf and latex manganese content.

No significant effects of manganese sulphate on the bark magnesium content were observed (RUBBER RESEARCH INSTITUTE OF MALAYA, 1961b).

Analysis of Bark Scrap Rubber

One application. Both the 4 oz and 8 oz applications caused marked increases in the manganese content of the bark scrap (*Figure 2*). The maximum manganese content of 58 p.p.m. (approximately four and a half times greater than the manganese content of the control) was observed sixteen months after the application of 8 oz. Thereafter a gradual decline, to approximately 20 – 30 p.p.m. was observed.

Two applications. The second application of both the 4 oz and the 8 oz manganese sulphate resulted in further increases in the manganese content.

The two 8 oz applications caused the greatest increase in bark scrap manganese, and a maximum content of 73 p.p.m. (approximately four and a half times greater than that of the control) was observed at seven months after the second application (*Figure 2*).

Soil Analysis

The total soil manganese content, as measured on the control plots was 19 p.p.m. Mn and the exchangeable manganese content was 0.0025 m-equiv./100 g. In *Table 6* the effects of the different rates of manganese sulphate application on both total and exchangeable manganese content are clearly shown. It is to be noted that the positive effect of manganese sulphate on the exchangeable soil

TABLE 6. EXPERIMENT 1. SOIL ANALYSIS

Date of sampling 4.3.61

	Total Mn p.p.m.	Exchangeable Mn m-equiv./100 g
Control	19	0.0022
Mn_1 4 oz 1957 only	23	0.0062
Mn_2 8 oz 1957 only	26	0.0073
s.e.	2.0	0.00055
Min. 5% sig. diff.	7.9	0.00222
Control	18	0.0029
Mn_1 4 oz 1957 and 1958	33	0.0116
Mn_2 8 oz 1957 and 1958	42	0.0135
s.e.	6.0	0.00193
Min. 5% sig. diff.	23.4	0.00765

manganese was evident three and four years after application.

EXPERIMENT 2

This experiment was adjacent to Experiment 1, on the same soil type and using the same planting material, with similar plot sizes, and was laid down to study the uptake of copper, molybdenum and manganese by the trees. For the purpose of this paper, only the manganese treatments, consisting of 0 and 4 oz/tree applications of manganese sulphate need to be considered. The application of manganese sulphate was carried out annually for three years.

RESULTS OF EXPERIMENT 2

Leaf Analysis

An increase in the leaf manganese content, significant at the 10% level, was observed at five months after the first application of 4 oz of manganese sulphate (*Table 7*). After the second application of manganese sulphate significant increases in leaf manganese content were recorded on all sampling occasions. Whilst a maximum leaf concentration of 165 p.p.m. manganese was recorded two weeks after the third 4 oz application, the effect of both the second and third application of manganese sulphate was to maintain the manganese content at a level of approximately four times greater than that of the control, an increase of the same order as the maximum observed in Experiment 1 (*Figure 1*) following two applications of 4 oz.

There were strong indications of a depressive effect of manganese sulphate on the leaf molybdenum content (*Table 7*), after the second application, and a significant interaction between manganese and molybdenum on uptake of molybdenum was observed, whereby the effect of sodium molybdate in increasing the leaf molybdenum content was considerably reduced in the presence of applied manganese.

Analysis of Bark Scrap Rubber

In this experiment the effect of one 4 oz application of manganese sulphate on the bark scrap manganese content appeared to be slightly greater than in Experiment 1 (*Table 7*). The effect became apparent after three months and a maximum mean concentration of 46 p.p.m. was recorded after five months.

EXPERIMENTS 3, 4 AND 5

These experiments are fertiliser experiments of factorial design, in which the application of 4 oz per tree of manganese sulphate is one treatment. Data on post-treatment leaf analysis are given in *Table 8* as confirmatory evidence of the effect of manganese sulphate on leaf manganese content.

TABLE 8. THE EFFECT OF MANGANESE SULPHATE ON THE MANGANESE CONCENTRATION IN THE LEAVES

Experiment No.	3	4	5
Time of sampling (months after application)	9	8	7
	p.p.m. Mn in dry lamina		
Control	47	90	43
Mn*	57	117	80
s.e.	1.6	8.9	4.2
Min. 5% sig. diff.	4.8	26.1	12.7

* See Table 1 for rates of application in each experiment.

TABLE 7. EXPERIMENT 2. THE EFFECT OF MANGANESE SULPHATE ON THE MANGANESE AND MOLYBDENUM CONCENTRATIONS IN THE LEAVES, AND ON THE MANGANESE CONCENTRATION IN THE BARK SCRAP RUBBER

Dates of Application: first, 11.6.58 (F1); second, 9.5.59 (F2); third, 23.4.60 (F3)

Dates of sampling	22.5.58 (F1)	20.6.58	18.7.58	11.9.58	8.11.58	20.4.59 (F2)	13.7.59	5.10.59 (F3)	4.5.60	5.8.60	2.11.60
					p.p.m. Mn in dry lamina						
Control	34	35	27	22	19	24	24	19	40	30	20
Mn 4 oz	27	26	21	24	28	84	73	76	165	111	80
s.e.	± 2.6	± 3.2	± 1.6	± 1.6	± 1.8	± 10.6	± 1.9	± 5.9	± 3.5	± 7.8	± 4.6
Min. 5% sig. diff.	N.S.	N.S.	N.S.	N.S.	N.S.*	N.S.*	11.7	35.8	21.2	48.0	27.8
					p.p.m. Mo in dry lamina						
Control	0.08	0.21	0.12	0.14	0.12	0.26	0.37	0.37	1.18	0.36	0.27
Mn 4 oz	0.08	0.23	0.15	0.15	0.13	0.27	0.32	0.24	0.97	0.24	0.19
s.e.	± 0.007	± 0.015	± 0.009	± 0.022	± 0.009	± 0.036	± 0.022	± 0.027	± 0.053	± 0.018	± 0.037
Min. 5% sig. diff.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.*	N.S.	0.109	N.S.

Dates of sampling (F1)	21.6.58	5.7.58	19.7.58	22.8.58	15.9.58	13.10.58	20.11.58	16.12.58	21.4.59 (F2)	24.7.59
					p.p.m. Mn in bark scrap rubber					
Control	11	11	9	12	13	15	19	14	14	15
Mn 4 oz	9	10	11	18	24	34	46	38	38	44

* Significant $P < 0.10$

DISCUSSION

At the time of commencement of the above experiments, the wide occurrence of manganese deficiency conditions in rubber growing areas was not fully appreciated. Information on methods of increasing the manganese status of trees was obviously required however, and Experiments 1 and 2 were sited on available areas of known low manganese status, although it was appreciated that the clonal variation present and small plot size would render difficult the measurement of response in terms of growth and latex yield. Nevertheless it is worth recording that in Experiment 2 a significant positive effect on growth of the manganese sulphate application was recorded in the second year of the experiment. In all experiments improvements in foliage colour have been observed following application of manganese sulphate.

Results show that two dressings of 4 oz of manganese sulphate, applied with a twelve month interval, were more effective than one application of 8 oz. The residual effect of two applications of 4 oz or 8 oz was quite definite two and a half years after the second application, whereas at the same time the leaf manganese content of trees treated with one application had declined to a level where deficiency symptoms could be expected. It is therefore judged that two applications of 4 oz manganese sulphate is the minimum required to improve the manganese status of mature trees affected by moderate to severe manganese deficiency. In cases of severe deficiency, where the leaf interveinal areas are yellow rather than pale green (BOLLE-JONES, 1956), two applications of 6 oz per tree would be preferable.

The increase in the manganese status of the trees was more clearly shown by the leaf and bark than by the bark scrap rubber, which in turn showed the effect more than did latex. The marked differences between the low manganese content of the latex and the high content of the bark scrap rubber is probably due to the fact that the bark scrap rubber remained in close contact with the bark, which has a high manganese content, for twenty-four hours. If collection of bark scrap rubber had been delayed for the full period of two days between tappings, the difference would probably have been greater.

The manganese content of the latex did not rise sufficiently to cause concern, but that of the bark scrap rubber, which was not soaked before analysis, rose well above the tolerance limit of 10 p.p.m. Under normal estate practice such bark scrap would be soaked and washed during milling, and the manganese content would be effectively reduced.

The general leaf analysis in Experiment 2 showed evidence of an antagonism between manganese and molybdenum similar to that reported by MULDER (1954), whereby applied manganese sulphate depressed the molybdenum content of the leaves. Except for this, no consistent interactions between the manganese content and that of the other nutrients have been recorded.

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The Rubber Research Institute of Malaya
Kuala Lumpur *June 1961*

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